

Decadal-scale litter manipulation alters the biochemical and physical character of tropical forest soil carbon

Daniela F. Cusack^{a,*}, Sarah M. Halterman^a, Edmund V.J. Tanner^b, S. Joseph Wright^c, William Hockaday^d, Lee H. Dietterich^a, Benjamin L. Turner^c

^a Department of Geography, UCLA, 1255 Bunche Hall, Los Angeles, CA 90095, USA

^b Department of Plant Sciences, U. of Cambridge, Cambridge CB2 3EA, UK

^c Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Panama

^d Department of Geosciences, Baylor U., 1 Bear Place, Waco, TX 76798, USA

ARTICLE INFO

Keywords:

Soil organic matter (SOM)

Nitrogen

Dissolved organic carbon (DOC)

¹³C NMR

Radiocarbon ¹⁴C

Density fractionation

ABSTRACT

Climate change and rising atmospheric carbon dioxide (CO₂) concentrations are likely to alter tropical forest net primary productivity (NPP), potentially affecting soil C storage. We examined biochemical and physical changes in soil C fractions in a humid tropical forest where experimental litter manipulation changed total soil C stocks. We hypothesized that: (1.) low-density soil organic C (SOC) fractions are more responsive to altered litter inputs than mineral-associated SOC, because they cycle relatively rapidly. (2.) Any accumulation of mineral-associated SOC with litter addition is relatively stable (i.e. low leaching potential). (3.) Certain biomolecules, such as waxes (alkyl) and proteins (N-alkyl), form more stable mineral-associations than other biomolecules in strongly weathered soils. A decade of litter addition and removal affected bulk soil C content in the upper 5 cm by +32% and −31%, respectively. Most notably, C concentration in the mineral-associated SOC fraction was greater in litter addition plots relative to controls by 18% and 28% in the dry and wet seasons, respectively, accounting for the majority of greater bulk soil C stock. Radiocarbon and leaching analyses demonstrated that the greater mineral-associated SOC in litter addition plots consisted of new and relatively stable C, with only 3% of mineral-associated SOC leachable in salt solution. Solid-state ¹³C NMR spectroscopy indicated that waxes (alkyl C) and microbial biomass compounds (O-alkyl and N-alkyl C) in mineral-associated SOC are relatively stable, whereas plant-derived compounds (aromatic and phenolic C) are lost from mineral associations on decadal timescales. We conclude that changes in tropical forest NPP will alter the quantity, biochemistry, and stability of C stored in strongly weathered tropical soils.

1. Introduction

Soil organic carbon (SOC) storage in humid tropical forests is likely to be affected by shifting net primary productivity (NPP) in response to global change. Remote sensing data show increased NPP for much of the tropics during recent decades in response to warming, increased rainfall (Nemani et al., 2003), and elevated atmospheric carbon dioxide (CO₂) (Lewis et al., 2009). At the same time, field studies have observed that warming (Clark et al., 2013) and drying (Tan et al., 2013) can suppresses NPP in tropical forests. This spatial variation may result from increased dry season duration in some areas (Boisier et al., 2015), versus increased wet season rainfall in other areas with climate change (Feng et al., 2013). Rainfall seasonality, in turn, is related to intra-annual shifts in SOC stocks, as shown for a humid forest in Panama (Turner et al., 2015). Since ~30% of global SOC stocks are in tropical

forests (Jobbagy and Jackson, 2000), potential interacting effects of altered NPP and seasonality are of broad significance.

Changes in NPP are most likely to affect SOC storage via altered litterfall and/or root turnover. A global meta-analysis of 70 litter addition experiments showed a 31% overall increase in SOC content to 5 cm depth, with the largest changes in six tropical or sub-tropical studies included in the study (Xu et al., 2013b). More recently, litter manipulation in a humid Panamanian forest also resulted in significant changes in SOC stocks to 20 cm depth (Tanner et al., 2016). However, we know very little about how altered litterfall might affect the biochemistry, physical character, and stability of tropical SOC.

A useful conceptual model divides SOC into three fractions (Sollins et al., 1999): (1) free-debris SOC, which visually resembles decomposing litter and roots, is unattached to mineral particles, and tends to have younger C than other fractions in tropical soils (Marin-Spiotta

* Corresponding author.

E-mail address: dcusack@geog.ucla.edu (D.F. Cusack).

<https://doi.org/10.1016/j.soilbio.2018.06.005>

Received 25 February 2018; Received in revised form 17 May 2018; Accepted 9 June 2018

Available online 23 June 2018

0038-0717/ © 2018 Elsevier Ltd. All rights reserved.

et al., 2008); (2) occluded-debris SOC, which is litter that has been incorporated into aggregate structures, or which is otherwise easily dispersible from soil; (3) mineral-associated SOC, often referred to as the “heavy fraction,” which consists of organic compounds sorbed to mineral surfaces via physiochemical mechanisms (Kleber et al., 2015). Studies of clay-rich tropical soils have found that the majority of SOC resides in the mineral-associated fraction (Trumbore, 1993; Glaser et al., 2000; Marin-Spiotta et al., 2009), which is generally thought to be the most stable, long-term SOC stock (Trumbore and Zheng, 1996; Torn et al., 1997; von Lutzow et al., 2006). Recent evidence, however, suggests that the mineral-associated SOC fraction can contain heterogeneous sub-fractions that turn over on different timescales (Sollins et al., 2006; Cusack et al., 2011b), with certain compounds like proteins comprising the most stable portion of mineral-associated SOC (Kleber et al., 2007). In humid tropical forests in particular waxy substances and other lipids may also contribute to more stable soil fractions (Cusack et al., 2011a).

Here, we investigate the biochemistry, physical character, and stability of SOC after a decade of litter manipulation in a humid Panamanian forest, following up on documented shifts in SOC stocks with litter addition or removal at these sites (Tanner et al., 2016). We measured three SOC fractions in surface soils, where the greatest changes in SOC stocks were documented, during a wet and a dry season. We hypothesized that: (1) changes in bulk SOC concentrations with litter manipulation are explained by shifts in rapidly cycling free-debris and occluded-debris SOC fractions, because these are likely to change over short timescales; (2) the quantity and chemical character of mineral-associated SOC is relatively insensitive to decadal-scale changes in litter inputs; (3) any new SOC accumulation in mineral-associated soil fraction with litter addition is relatively stable (i.e., low solubility); (4) waxy (alkyl) and protein-rich (*N*-alkyl) C groups represent the most stable portion of mineral-associated SOC, persisting on decadal timescales even in the absence of new litter inputs. Finally, (5) seasonal shifts in SOC result primarily from changes in the free-debris and occluded-debris SOC fractions.

2. Materials and methods

2.1. Site and soil collection

Research was conducted in a lowland semi-deciduous tropical forests on the Gigante Peninsula in the Barro Colorado Nature Monument, Panama (9°06'N, 79°54'W) (Windsor, 1990). The forest is a tropical moist forest *sensu* Holdridge et al. (1971). The site has a tropical monsoon seasonal climate, mean annual rainfall is 2600 mm, and mean annual temperature is 26°C (Leigh, 1999). High tree species richness and the stature (canopy heights > 35 m) of this forest are characteristic of an old (> 200 years) seasonal primary forest (Leigh et al., 1996). Litterfall peaks during the dry season from December to April, and decreases by 50% during the wet season (Turner et al., 2015). Soils are moderately acidic (pH 4.8–5.4) Oxisols developed on basalt (Yavitt et al., 2009). The soils lack a distinct O horizon other than a continuous cover of leaf litter that builds up during the dry season and is virtually fully decomposed by the end of the wet season. Soils were classified in profile pits outside of plots according to Soil Taxonomy (Soil Survey Staff, 1999). Soil were well-structured, with medium and coarse sub-angular blocky structure in the upper parts of the profile, breaking to strong fine subangular blocky. The clay mineralogy is kaolinitic.

Fifteen 45 × 45 m litter manipulation plots were established in 2000 and trenched to a depth of 50 cm. The sides of the trenches were lined with heavy plastic. This depth was chosen to contain fine roots and deter growth into or out of plots, since > 92% of fine root biomass is in the top 50 cm of soil in forests in this region (Yavitt and Wright, 2001; Cusack et al., 2018). Monthly transfer of litter from five removal plots to five addition plots began in January 2003, and five control plots were included (*n* = 5). Litter was removed by rake and by hand

monthly and evenly distributed onto the litter addition plots. A study to 1-m depth after 6 years of litter manipulation at these sites showed that litter addition and removal significantly increased and decreased bulk soil C stocks to 20 cm depth, respectively, with the greatest effect in the upper 5 cm of the profile (Tanner et al., 2016). This was consistent with an earlier study at 3 years showing significant treatment effects on organic matter concentrations in the top 2 cm of soils at the site (Vincent et al., 2010). Our goal was to better understand the physiochemical nature of the changing SOC stocks documented in these previous studies.

Soils for this study were collected during the dry season in February 2013, and the wet season in July 2013. Soils were sampled from the surface 5 cm using a 2.5 cm diameter constant-volume soil corer, thus sampling equivalent depths and volumes in all treatments. Surface litter was gently removed and coring began at the surface of the mineral soil. In litter addition plots a spatially heterogeneous duff layer had developed, and this was included in sampling where it was present. Three soil cores were sampled from 10 random stratified points within the 30 × 30 m core area of each plot, then pooled and homogenized to give one representative sample per plot. The 0–5 cm depth was selected because it showed the strongest changes in bulk soil C with litter manipulation at 6 years (Tanner et al., 2016), and this depth has shown the strongest changes in litter manipulation experiments more broadly (Xu et al., 2013a).

Bulk density cores were collected using a 5 cm diameter corer in each plot. Gravimetric soil moisture, pH, bulk density, and SOC fractions (see below) were measured on fresh soils. Loss on ignition (LOI) was also measured for dry season samples to assess changes in the mineral content of soils across treatments to the same depth. Carbon content normalized to mineral mass was assessed as an alternative to the strategy used here of collecting samples from a constant depth and volume across plots.

Given the previously documented change in bulk density with litter removal at these sites (Tanner et al., 2016), an alternative sampling approach could have been to adjust sampling depth in an attempt to collect equivalent mass. Soil volume increases and bulk density declines as organic matter is added. Thus, we would have had to sample to shallower depths in litter removal plots and deeper depths in litter addition plots to collect equivalent mass. This would pose problems because the composition of SOC changes with depth. For example, the proportion of dense, mineral-associated SOC increases with depth. We therefore determined that sampling equivalent mass would have masked any increased importance of low-density organic matter in surface soils, as reviewed by Sollins and Gregg (2017). Since our goal was to characterize the physiochemical characteristics of the change in surface SOC including chemical shifts related to changes in bulk density, we decided to sample to constant depth and volume rather than correct for changes related to bulk density.

2.2. Soil fractionation

Density fractionation was used to separate soils into three soil SOC pools using a solution of C-free sodium polytungstate (SPT) following Swanston et al. (2005), with modifications as noted. This method was chosen over particle size fractionation because it puts greater emphasis on adsorption and aggregation processes, rather than the fate of particulate organic matter (Moni et al., 2012). The density fractions included: (1) free-debris SOC, which was collected as the fraction of the soil that easily floated in dense liquid with minimal disturbance (sometimes referred to as the “free light fraction” in the literature). (2) Occluded-debris SOC, which was collected as floatable SOC that was released from soil via mixing and dispersed via sonication at 450 J/mL (sometimes referred to as the “occluded light fraction”). This SOC fraction is generally considered to turnover more slowly than the free-debris SOC fraction because of its physical protection with aggregate structures, as shown in an Australian Oxisol (Golchin et al., 1995). This

fraction may also represent SOC at more advanced stages of decomposition than the free-debris, as suggested by elevated alkyl:O-alkyl ratios (Golchin et al., 1994). However, the quantity and chemistry of SOC released via sonication can be highly variable across soils (Wagai et al., 2009), and may include debris buried inside macro-aggregates, debris entrained in the organic coatings that have built up at the surface of micro-aggregates, and/or individual organic particles released from mineral grains by sonication. Iron-oxide particles in Oxisols are often bound up in exceptionally stable micro-aggregates ($< 5 \mu\text{m}$), and these were not likely broken down by the method used here. (3) Mineral-associated SOC, which was collected as the dense pellet of soil remaining after removing the above two fractions (sometimes referred to as the “dense” or “heavy” fraction). This fraction generally represents the most stable and oldest soil C. Separation of fractions was done in sodium polytungstate (SPT) mixed to a density of 1.7 g/cm^3 , following initial tests to identify optimal density and dispersal parameters (Appendix A, S1).

Bulk soils and SOC fractions were analyzed for C and N concentrations on a Costech Elemental Analyzer (Valencia, CA) using acetanilide as a standard. While the focus of this paper is on SOC, we included parallel N analyses to understand broader effects of litter manipulation on soil organic matter (SOM) N content and C:N ratios, particularly in relation to SOM protein content versus other C functional groups.

2.3. $\Delta^{14}\text{C}$ and ^{13}C NMR of mineral-associated SOC

Because the mineral-associated SOC fraction commonly contains the longest-term soil C sink (Sollins et al., 1999), we conducted extensive chemical analysis of this C fraction to determine the potential effects of changing NPP on long-term tropical soil C storage.

First, we measured the ^{14}C content of the mineral-associated SOC fraction to assess relative changes in the overall age of this C fraction with litter addition and removal. The spike in atmospheric $^{14}\text{CO}_2$ from nuclear bomb testing in the 1960s and subsequent declines make it possible to assess relative ages of plant organic matter inputs to ecosystems on decadal timescales (Trumbore, 2000). The mineral-associated SOC fraction was measured for $\Delta^{14}\text{C}$ at the WM Keck Carbon Cycle Accelerator Mass Spectrometry (AMS) Laboratory at UC Irvine following sample graphitization (Vogel et al., 1984). AMS analytical precision was $\pm 2.2\text{‰}$ for these samples, and results are reported as the per mil (‰) deviation from a standard normalized for ^{13}C .

We also assessed changes in the organic chemistry of the mineral-associated SOC fraction to understand how litter addition and removal altered this important C fraction. The organic chemistry of the mineral-associated fraction was assessed using solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy following hydrofluoric acid treatment (details in Appendix A, S1), which removes mineral and glass fractions to reduce magnet interference, and concentrates SOC to reduce noise in the spectra (Schmidt et al., 1997). This method also removes a portion of acid-soluble C as DOC from mineral associations (Gelinas et al., 2001), leaving a sample of mineral-free, concentrated organic matter that is not soluble in hydrofluoric acid. Here, the hydrofluoric-acid-insoluble portion of mineral-associated SOC was 60.5% of the C contained in the mineral-associated fraction (Appendix A, Table S5), which is within the range found for a comparison of different soil types (Hockaday et al., 2009). The mineral-associated SOC remaining after hydrofluoric acid treatment is the least dynamic, least soluble component of this pool.

Cross polarization ^{13}C NMR was conducted on the concentrated SOC after hydrofluoric acid treatment using a 300 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin, Billerica, MA) at Baylor University (Waco, TX). The 4 mm sample probe was operated at a magic angle spinning (MAS) frequency of 12 kHz (details in Appendix A, S1). Resulting spectra were assessed for the relative contributions of seven C functional groups by integrating the signal magnitude on the δ -scale.

Carbon functional groups are followed in parentheses by some of the many common C compounds in which they occur: (1.) alkyl 0–45 ppm (e.g., waxes, other lipids); (2.) N-alkyl + methoxyl (hereafter N-alkyl) 45–60 ppm (e.g., proteins, peptides); (3.) O-alkyl 60–95 ppm (e.g., cellulose, other carbohydrates); (4.) di-O-alkyl 95–110 ppm (e.g., hemicellulose); (5.) aromatic 110–145 ppm (e.g., lignin, tannin); (6.) phenolic 145–165 ppm (e.g., acids, tannin (Min et al., 2015)); and (7.) amide + carboxyl 165–215 ppm (e.g., chitin, proteins, peptides, and hemicellulose) (Li et al., 2015). The alkyl:O-alkyl ratio was calculated as an index of decomposition stage, with greater ratios generally indicative of later stages of litter decomposition (Preston et al., 2009).

2.4. Soil C stability and DOC leaching

The stability of C was assessed in each SOC density fraction by measuring the solubility of organic matter as DOC and TDN leached in the SPT supernatant plus approximately 1-L of deionized water (DI). This approach specifically mobilized DOC from cation and anion exchange sites, in an analogous approach to the commonly used potassium chloride (KCl) salt extractions for collecting DOC from soil (Swift, 1996). Floatable debris fractions were rinsed with approximately 1-L of DI through a $0.4 \mu\text{m}$ membrane filter, which both cleaned the fraction of SPT, and allowed us to conduct the leaching experiment. The dense fraction was rinsed with DI in centrifuge tubes by shaking, centrifuging, decanting of supernatant. Decanted supernatant was then filtered as above, and any residue on the filter was added back to the dense fraction. Initial rinses included removal of the SPT from the fraction. Thus, all C in initial bulk soils was accounted for either in SOC solid fractions, or in leachate from the fractionation procedure and subsequent leaching (details in Appendix A, S1). Leachate samples were analyzed on a Shimadzu TOC-L with TDN analyzer (Columbia, MD).

2.5. Statistical analyses

Data from the litter manipulation plots were analyzed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The effects of season, treatment, and their interaction were tested for bulk soil C and N content, C and N in each density fraction, ^{14}C content of the mineral-associated fraction, and for ^{13}C NMR chemical regions of the mineral-associated fraction. Effects of season and treatment were also tested for DOC and TDN leached from each SOC fraction. We used post-hoc Tukey HSD means separation tests to assess differences among litter treatments and seasons ($n = 5$ per season and litter treatment). Post-hoc regressions were used to determine relationships between continuous variables across seasons ($n = 30$ for bulk soils, 30 for each density fraction). We used JMP 11 statistical software (SAS Institute). Data are presented as means ± 1 S.E., using $p < 0.05$ for statistical significance.

3. Results

After 10 years of litter manipulation, bulk soil C concentrations from 0 to 5 cm depths were significantly greater with litter addition (+32%), and significantly lesser with litter removal (−31%) relative to control plots (Fig. 1a, Table 1). Similar significant effects were observed for soil N concentrations, but the magnitude of the differences were not as large, so soil C:N values were significantly affected by treatment, with the lowest levels in litter removal plots (Table 1).

Bulk density was also significantly greater in litter removal plot ($0.97 \pm 0.05 \text{ g/cm}^3$) relative to the other treatments, while there was no significant difference between control ($0.73 \pm 0.03 \text{ g/cm}^3$) and litter addition plots ($0.79 \pm 0.05 \text{ g/cm}^3$, $n = 5$), and no seasonal effect (Table 1). This change represents a relatively large (+28%) increase in bulk density with litter removal relative to the other two treatments. Our approach of sampling equivalent depths and volumes across treatments explicitly did not correct for changes in bulk density, since

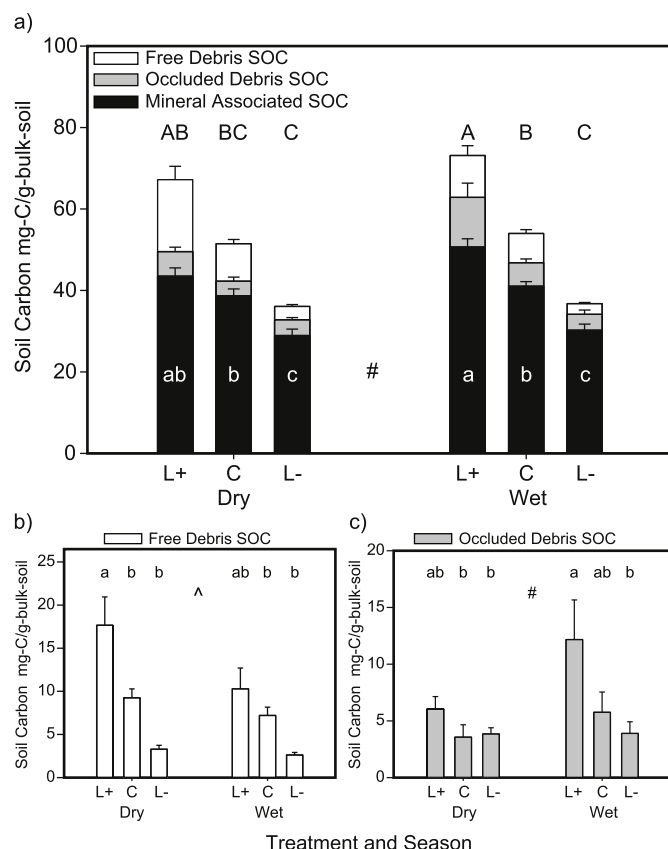


Fig. 1. (A.) Bulk soil C concentrations, made up of three SOC fractions, are shown for litter addition (L+), control (C), and litter removal (L-) plots during a wet and dry season (0–5 cm depth). Bulk soil C concentrations were significantly greater in L+ versus C and L-, driven primarily by greater mineral-associated SOC. (B.) Free-debris SOC was greater in L+ versus C and L-, with the largest treatment effect during the dry season. (C.) Occluded-debris SOC was greater in L+ versus L-, with the largest treatment effect during the wet season. ^ indicates significantly greater free-debris SOC during the dry season; # indicates significantly greater mineral-associated and occluded-debris SOC fractions during the wet season. Tukey HSD means separation results are shown in capital letters for bulk soil C concentrations, and lower case letters for SOC fractions, showing differences across both seasons. Averages are shown for each fraction \pm one SE for that fraction ($n = 5$).

these changes were due to addition of low-density organic matter, which we did not want to dilute by sampling to greater depth. Nonetheless, we can evaluate the effect of the change in bulk density on our bulk soil C content values. Using published data from our sites for C concentration versus depth to 200 cm at six years (Tanner et al., 2016), we can calculate the depths we would have had to sample, and the resulting effect on bulk soil C content. The 28% greater bulk density

that we observed with litter removal relative to controls is equivalent to a 1.4 cm increase in effective soil depth. Using the published soil depth profiles, we calculate that this greater effective soil depth would lead to a 2.4% increase in C for litter removal plots relative to control plots, if our goal had been to calculate C stocks on a per-mass of soil basis. This is a small proportion of our observed 31% decline in bulk soil C concentration with litter removal.

Another approach is to normalize soil C content to mineral content. Loss on ignition values were significantly lower in litter removal plots ($17.5 \pm 0.3\%$) relative to other treatments, indicating greater content of minerals/ash, and there was no significant difference between control and litter addition plots ($19.7 \pm 0.3\%$ and 20.6 ± 0.8 , respectively, $n = 5$). Using these LOI values and bulk density to standardize soil C content to mineral content for 0–5 cm depth, there was significantly greater soil C in litter addition plots (98.5 ± 4.5 mg-C/g-mineral-content), intermediate levels in control plots (75.3 ± 3.0 mg-C/g-mineral-content), with lower values in litter removal plots (49.5 ± 2.5 mg-C/g-mineral-content; $p < 0.05$). Thus, normalizing soil C to mineral content gave similar differences between addition vs. control (+31%), and removal vs. control (–34%) as did C concentration and C stock data sampling equivalent volume (Table 1).

3.1. Soil fraction C contents, relative masses, and C concentrations

Soil organic C content was greater with litter addition versus controls and litter removal for all three soil fractions (Fig. 1), with accompanying changes in C concentrations within each fraction (Appendix A, Table S2), and changes in the relative masses of the three fractions (Fig. 5a), such that the contribution of each fraction to total soil C shifted significantly with litter manipulation (Fig. 5b). There were also seasonal effects on all fractions, with interactions between season and treatment effects. The most important effect of litter manipulation on the mineral-associated SOC fraction, in terms of the net effect on bulk soil C content, was a change in C concentration within this fraction. In contrast, the most important effect on the free-debris and occluded-debris SOC fractions was an increase in the relative mass of these fractions as a proportion of total soil mass with litter addition. Results for the mineral-associated fraction are presented first, followed by the two floatable fractions together.

3.1.1. Mineral-associated SOC fraction

The mineral-associated SOC fraction comprised the largest portion of greater bulk soil C with litter addition (Fig. 1a). The changes in mineral-associated soil C content resulted from greater C concentrations in this soil fraction with litter addition, and lesser C concentrations with litter removal (Appendix A, Table S2). Even though average C concentrations for the mineral-association SOC fraction were low relative to the other two fraction (3–5% C in mineral-association SOC, versus 20–40% C in the floatable fractions, Appendix A, Table S2), the large relative mass of this soil fraction (91–97% of soil mass, Fig. 5a)

Table 1

Effects of litter manipulation on bulk soil chemistry and soil characteristics are shown for a wet and a dry season in Panama (0–5 cm depth). Averages are given \pm one SE ($n = 5$).

Season	Treatment	Bulk Soil %C ^a	Bulk Soil %N ^a	Bulk Soil C:N ratio ^a	Soil Moisture g-water/g-soil ^{a, b, c}	Bulk Density (g/cm ³) to 5 cm depth ^a	C Stock (mg/cm ²) to 5 cm depth ^a	Leachable DOC mg-C/g-soil ^a	Leachable TDN μ g-N/g-soil ^a
Dry	Addition	6.7 \pm 0.5	0.63 \pm 0.02	10.7 \pm 0.8	0.43 \pm 0.03	0.82 \pm 0.08	269 \pm 20	1.1 \pm 0.3	254 \pm 38
	Control	5.2 \pm 0.3	0.52 \pm 0.02	9.9 \pm 0.3	0.42 \pm 0.01	0.78 \pm 0.03	200 \pm 14	0.9 \pm 0.1	213 \pm 40
	Removal	3.6 \pm 0.2	0.38 \pm 0.01	9.4 \pm 0.4	0.35 \pm 0.01	0.99 \pm 0.04	180 \pm 15	0.5 \pm 0.04	128 \pm 5
Wet	Addition	7.3 \pm 0.6	0.61 \pm 0.04	12.0 \pm 0.8	0.72 \pm 0.02	0.76 \pm 0.02	278 \pm 25	1.6 \pm 0.3	227 \pm 35
	Control	5.4 \pm 0.3	0.51 \pm 0.02	10.5 \pm 0.3	0.66 \pm 0.02	0.68 \pm 0.03	184 \pm 15	0.9 \pm 0.1	150 \pm 15
	Removal	3.7 \pm 0.2	0.38 \pm 0.02	9.6 \pm 0.4	0.52 \pm 0.01	0.95 \pm 0.06	175 \pm 16	0.6 \pm 0.1	111 \pm 17

^a Significant effect of treatment.

^b Significant effect of season.

^c Interacting effect of treatment and season.

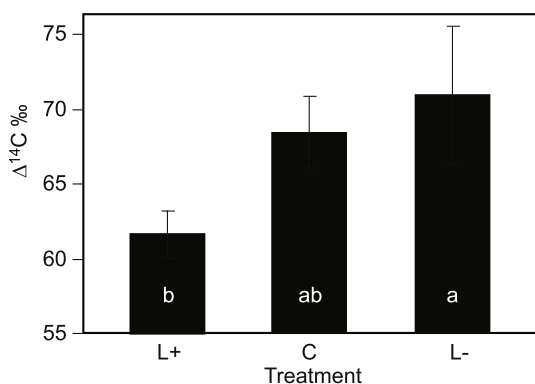


Fig. 2. Average radiocarbon ($\Delta^{14}\text{C}$) values for mineral-associated SOC are shown for litter addition (L+), control (C), and litter removal (L-) plots. All $\Delta^{14}\text{C}$ values reflect ^{14}C released during bomb testing in the 1960s (i.e., “modern” values), so lesser $\Delta^{14}\text{C}$ values in L+ versus L- indicate more retention of recent C inputs into mineral-associated SOC with litter addition. Letters shown in the bars indicate Tukey HSD differences in the mean among the treatments. There were no seasonal effects so values were pooled. Averages are shown \pm one SE ($n = 5$).

meant that it dominated net changes in soil C content. So, even with the relatively low average C concentrations, the mineral-association SOC fraction contributed the largest overall proportion of soil C content, containing 64–82% of total soil C (Fig. 5b).

There was also a seasonal effect on C concentrations within the mineral-associated fraction, with greater C concentrations overall and larger differences among treatments during the wet season (Fig. 1a). Specifically, mineral-associated C concentrations were $4.0 \pm 0.2\%$ in control plots versus $4.7 \pm 0.2\%$ in litter addition plots during the dry season (Appendix A, Table S2), representing an 18% increase. During the wet season, mineral-associated C concentrations were $4.3 \pm 0.1\%$ in control plots, versus $5.5 \pm 0.2\%$ in litter addition plots (Appendix A, Table S2), representing a 28% increase in C concentration. Mineral-associated C concentrations in litter removal plots were 25% lower than control plots the dry season, and 28% lower than control plots during the wet season (Appendix A, Table S2). Nitrogen concentrations in the mineral-associated soil fraction showed similar significant shifts with litter removal, but the magnitude of changes in N concentration were not as large, so C:N ratios in the mineral-associated SOC fraction were lower in litter removal plots than in litter addition plots, indicating a relative enrichment in N with litter removal (Appendix A, Table S2).

Assessing the total C content within the mineral-associated fraction, using C concentrations and the mass of this fraction as a proportion of soil mass, there was 43.5 ± 2.1 mg-C/g-bulk-soil in mineral-associated SOC in litter addition plots, 38.7 ± 1.7 mg-C/g-bulk-soil in control plots, and 28.9 ± 1.6 mg-C/g-bulk-soil in litter removal plots in the dry season (Fig. 1a). In the wet season there was 50.7 ± 2.0 mg-C/g-bulk-soil in the mineral-associated SOC fraction in litter addition plots, 41.0 ± 1.1 mg-C/g-bulk-soil in control plots, and 30.2 ± 1.5 mg-C/g-bulk-soil in litter removal plots (Fig. 1a). Thus, litter addition plots had 12% greater mineral-associated SOC content than controls during the dry season, and 24% greater during the wet season relative to controls. Litter removal plots showed less of a seasonal effect on mineral-associated SOC content, with removal plots 25% lesser than controls during the dry season, and 26% lesser during the wet season relative to controls. Comparing shifts from the dry season to the wet season, mineral-associated SOC content increased 17% in the litter addition plots from the dry to the wet season, 6% in control plots, and only 4% in litter removal plots. These seasonal shifts illustrate the interaction between litter treatment and season, with wet season increases in mineral-associated SOC content much greater in litter addition versus litter removal plots.

3.1.2. Low-density SOC fractions

Unlike the mineral-associated SOC fraction, the change in soil C content attributable to the two floatable fractions (Fig. 1b and c) was related primarily to shifts in their relative masses within soil (Fig. 5a), rather than large changes in their C concentrations (Appendix A, Table S2). Nonetheless, there were significant but small effects of litter manipulation and season on C concentration in the free-debris SOC fraction (Appendix A, Table S2). The occluded-debris fraction had no significant effect of litter manipulation on C concentrations.

The shift in the relative masses of these fractions with litter manipulation was quite large. For the free-debris SOC fraction, relative mass was 89% and 37% greater in litter addition plots relative to controls during the dry and wet seasons, respectively (Appendix A, Table S2, Fig. 5a), and -63% and -58% lesser in litter removal plots relative to controls during the dry and wet seasons, respectively. The occluded-debris SOC fraction did not show strong treatment effects during the dry season, but during the wet season its mass was 74% greater in litter addition plots relative to controls, and -18% lower in litter removal plots relative to controls (Appendix A, Table S2, Fig. 5a). Given the sizeable shifts in the proportional masses of the two low-density fractions with litter manipulation, and their large C concentrations, their overall contributions to total soil C content was significant, even though their proportional masses were small, contributing only 1–6% of total soil mass (Appendix A, Table S2).

Using C concentration values and soil mass proportions to calculate the C content in the two low-density SOC fractions, the free-debris SOC fraction had 17.7 ± 3.4 mg-C/g-bulk-soil in litter addition plots, 9.2 ± 1.0 mg-C/g-bulk-soil in control plots, and 3.4 ± 0.5 mg-C/g-bulk-soil in litter removal plots in the dry season (Fig. 1b). In the wet season, there was 10.3 ± 2.4 mg-C/g-bulk-soil in litter addition plots, 7.2 ± 1.0 mg-C/g-bulk-soil in control plots, and 2.6 ± 0.3 mg-C/g-bulk-soil in litter removal plots for the free-debris SOC fraction (Fig. 1b). This represents 92% greater free-debris C content in litter addition plots versus controls in the dry season, and 43% greater free-debris C content in litter addition plots versus controls during the wet season, as well as 63% less in litter removal plots versus controls during the dry season, and 64% less in litter removal plots versus controls during the wet season.

In the occluded-debris SOC fraction, there was 6.1 ± 1.1 mg-C/g-bulk-soil in litter addition plots, 3.6 ± 1.1 mg-C/g-bulk-soil in control plots, and 3.8 ± 0.5 mg-C/g-bulk-soil in litter removal plots in the dry season (Fig. 1a). In the wet season, there was 12.1 ± 3.5 mg-C/g-bulk-soil in litter addition plots, 5.8 ± 1.8 mg-C/g-bulk-soil in control plots, and 3.9 ± 1.0 mg-C/g-bulk-soil in litter removal plots for the free-debris SOC fraction (Fig. 1c). This represents 69% more occluded-debris C in litter addition plots versus controls during the dry season, and 108% more occluded-debris C in litter addition plots versus controls during the wet season. There was no significant decrease in occluded-debris C content with litter removal in the dry season, but there was 33% less occluded-debris C content in litter removal plots versus controls during the wet season.

There were also overall seasonal effects on the proportion of soil mass and the proportion of C content for the two low-density SOC fractions. The free-debris SOC fraction was a larger proportion of total soil C (Fig. 5b) and of total soil mass (Fig. 5a, Appendix A, Table S2) during the dry versus wet season. In contrast, the occluded-debris fraction represented a significantly larger proportion of total soil C (Fig. 5b) and of total soil mass (Appendix A, Table S2) during the wet versus dry season. Thus, seasonal patterns for C content in the occluded-debris fraction mirrored patterns for the mineral-associated SOC fraction, and the occluded-debris fraction had opposite seasonality (Fig. 1).

3.2. Organic chemistry and $\Delta^{14}\text{C}$ age of mineral-associated SOC

The $\Delta^{14}\text{C}$ of the mineral-associated SOC fraction was significantly greater with litter removal versus addition, with no seasonal effect

Table 2

The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for the mineral-associated SOC fraction in litter addition, control, and litter removal plots. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. There were no seasonal effects so data are pooled for two seasons. Averages are given \pm one SE ($n = 5$).

Plant Litter Treatment	Alkyl C (waxes, other lipids) 0–45 ppm ^a	N-Alkyl + Methoxyl C (proteins, peptides) 45–60 ppm ^a	O-Alkyl C (cellulose, other carbohydrates) 60–95 ppm ^a	Di-O-Alkyl C (hemicellulose) 95–110 ppm ^{a, b}	Aromatic C (lignin, tannin) 110–145 ppm ^a	Phenolic C (acids, tannin) 145–165 ppm ^a	Amide + Carboxyl C (chitin + hemicellulose) 165–215 ppm ^b	Ratio of (Alkyl + O-alkyl + N-alkyl): (phenolic + aromatic) C ^a
Addition	25.3 \pm 0.5	9.2 \pm 0.2	18.2 \pm 0.4	7.3 \pm 0.3	17.4 \pm 0.4	7.9 \pm 0.2	14.7 \pm 0.5	2.1 \pm 0.08
Control	25.4 \pm 0.3	9.7 \pm 0.2	19.5 \pm 0.3	7.0 \pm 0.2	16.4 \pm 0.3	7.2 \pm 0.1	14.8 \pm 0.4	2.3 \pm 0.06
Removal	28.4 \pm 0.5	10.3 \pm 0.2	21.1 \pm 0.5	6.4 \pm 0.2	14.3 \pm 0.4	5.9 \pm 0.3	13.6 \pm 0.4	3.0 \pm 0.1

^a Significant effect of litter manipulation.

^b While cellulose and hemicellulose are both examples of carbohydrates, which are built from O-Alkyl C, hemicellulose contains carboxyl C and can thus be distinguished in NMR spectra. Glucuronic acid is a major constituent of hemicelluloses in gymnosperms and angiosperms, and glucuronic acid contains carboxyl C. The N-Acetyl glucosamine polymer (chitin) is the primary cell wall component of fungi, and as the name indicates, chitin contains acetyl groups. Acetyl C is a type of carboxyl C. Therefore, both hemicellulose and chitin contain carboxyl C in measurable quantities. Hemicellulose and chitin are both carbohydrates and both cell wall components of abundant organisms in the ecosystem.

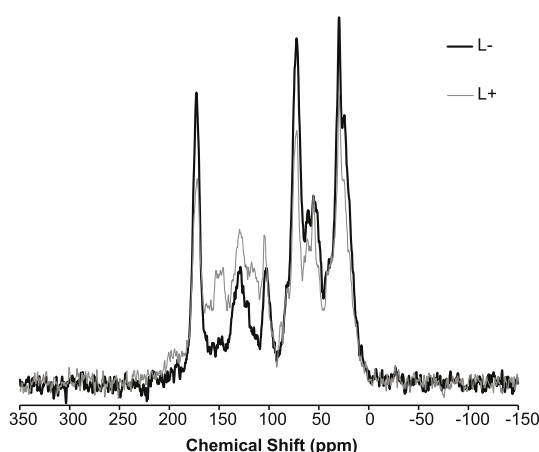


Fig. 3. Representative ^{13}C NMR spectra are shown for mineral-associated SOC in a litter removal plot (black line, L-), and a litter addition plot (gray line, L+). Chemical shift regions shown on a unitless δ -scale represent seven SOC functional groups: alkyl (0–45 ppm), N-alkyl + methoxyl (45–60 ppm), O-alkyl (60–95 ppm), di-O-alkyl (95–110 ppm), aromatic (110–145 ppm), phenolic (145–165 ppm), and amide + carboxyl (165–215 ppm). The proportion of total SOC in the alkyl, N-alkyl and O-alkyl regions was significantly after a decade of litter removal versus addition (Table 2).

(Fig. 2). Because the $\Delta^{14}\text{C}$ signal in surface soil is dominated by recent C inputs from plant litter, the greater values in litter removal plots correspond to an older overall age of the mineral-associated SOC fraction, versus a younger overall mineral-associated SOC fraction in litter addition plots with greater dominance by recent litter inputs.

Solid-state ^{13}C NMR spectroscopy revealed decadal-scale effects of litter manipulation on the organic chemistry of mineral-associated SOC, with no seasonal effects. All C groups responded to litter manipulation except amide + carboxyl C. Overall, alkyl C, N-alkyl C, and O-alkyl C were proportionally larger components of mineral-associated SOC in litter removal versus addition plots, while di-O-alkyl C, aromatic C, and phenolic C showed the opposite pattern (Table 2). These changes resulted in an overall shift in the chemical nature of mineral-associated SOC, as illustrated by comparing representative spectra from litter addition and removal plots (Fig. 3). Thus, there was a significant treatment effect on the ratio of (alkyl + O-alkyl + N-alkyl):(phenolic + aromatic) C (Table 2). There was also a significant interaction between season and treatment only for the alkyl:O-alkyl ratio in mineral-associated SOC, with significantly greater ratios in litter addition plots (1.5 ± 0.04) versus control (1.4 ± 0.03) and removal plots (1.3 ± 0.02) during the dry season only, and no effect during the wet season. This dry season pattern is the opposite pattern of what would be

expected for more advanced stages of decomposition in litter removal plots. Overall, these alkyl:O-alkyl ratio for mineral-associated SOC were all significantly greater than values obtained for occluded-debris SOC at varying sonication energy levels, suggesting that our method appropriately separated these two fractions (Appendix A, S1).

Two C chemical groups were significantly related to the $\Delta^{14}\text{C}$ content of mineral-associated SOC. Mineral-associated $\Delta^{14}\text{C}$ was positively significantly correlated to O-alkyl C ($R^2 = 0.18$, $n = 30$), indicating older C present when more O-alkyl C was present. In contrast, mineral-associated $\Delta^{14}\text{C}$ was negatively significantly correlated to phenolic C ($R^2 = 0.27$, $n = 30$), indicating younger C present when more phenolic C was present.

3.3. SOC stability: DOC leaching

The proportion of salt-extractable C (i.e., leachable DOC) in each soil fraction, expressed as mg-C-leached/g-C retained, varied across SOC fractions, with no effect of season. Overall, DOC leaching was 5–14% of the stable C in the free-debris (“free floatable”) fraction, 15–30% of the stable C in the occluded-debris (“dispersible floatable”) fraction, and only 2–3% of the stable C in the mineral-associated (“dense”) fraction (Fig. 4, Appendix A, Table S3). Thus, all SOC fractions had a component that was salt-extractable, but this proportion was smallest in the mineral-associated fraction, indicating that more of the C in this fraction was relatively stable.

The effect of litter manipulation on the proportion of C that was salt-extractable varied across SOC fractions. In the free-debris fraction, a significantly greater proportion of DOC was leachable from litter removal plots ($12.7 \pm 0.8\%$) relative to control ($6.2 \pm 0.6\%$) or litter addition plots ($4.9 \pm 0.4\%$), suggesting a high level of instability and solubility for the small stock of free-debris organic matter remaining in litter removal plots after a decade (Fig. 4a). In contrast, the mineral-associated SOC fraction had a significantly greater proportion of leachable DOC in litter addition plots ($2.9 \pm 0.3\%$), relative to control ($2.3 \pm 0.2\%$) or litter removal plots ($1.8 \pm 0.1\%$, Fig. 4b), indicating that C remaining in mineral associations in litter removal plots after a decade was strongly sorbed to mineral surfaces and stable. Significant treatment effects on proportional losses of total dissolved N (TDN) were similar to those for DOC, although N was much more mobile overall than C (Appendix A, Table S3).

Overall, the proportion of C that was leachable as DOC from the mineral-associated fraction was positively correlated with the total quantity of C in that fraction ($R^2 = 0.66$, $p < 0.05$, $n = 30$). Accordingly, bulk quantities of leachable DOC per g soil showed similar treatment effects as did soil C concentrations across fractions (Appendix A, Fig. S6 versus Fig. 1, Appendix A, Table S2), and so did trends for leachable TDN per g soil (Table 2, Appendix A, Table S4). Total DOC

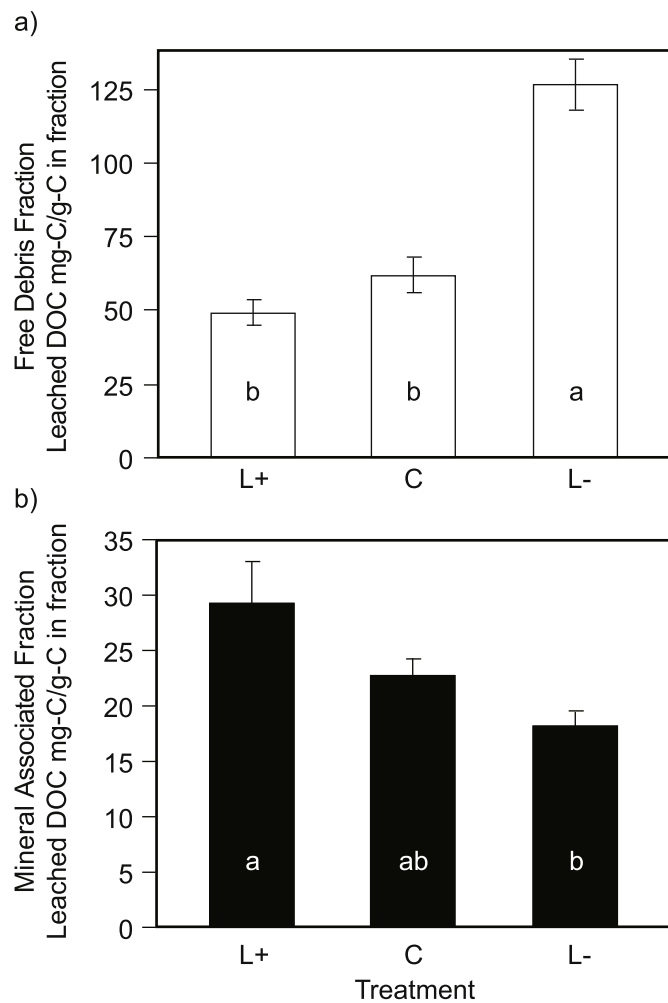


Fig. 4. The proportion of C leached as DOC is shown for (A.) free-debris SOC, and (B.) mineral-associated SOC, comparing litter addition (L+), control (C), and litter removal (L-) plots. (A.) A greater proportion of free-debris SOC was leachable as DOC in L-versus L+. (B.) In contrast, a greater proportion of the mineral-associated SOC was leachable as DOC in L+ versus L-, suggesting greater C desorption. Letters indicate Tukey HSD differences in the mean among the treatments. There were no seasonal effects so values were pooled. Averages are shown \pm one SE ($n = 5$).

leaching was 1.1 ± 0.2 mg-C/g-bulk-soil in litter addition plots, 0.9 ± 0.1 mg-C/g-bulk-soil in control plots, and 0.5 ± 0.04 mg-C/g-bulk-soil in litter removal plots in the dry season (Fig. 1a). In the wet season DOC leaching was 1.6 ± 0.3 mg-C/g-bulk-soil in litter addition plots, 0.9 ± 0.1 mg-C/g-bulk-soil in control plots, and 0.6 ± 0.1 mg-C/g-bulk-soil in litter removal plots (Appendix A, Fig. S6). These quantities represent very small fractions of total soil C content (Fig. 1), ranging from just 1.7–3.1% of total soil C leachable as DOC.

4. Discussion

The effects of litter manipulation on bulk soil C concentrations after 10 years in this tropical forest indicate a sustained effect, with similar effects observed from 0 to 2 cm depth after three years (Vincent et al., 2010), and from 0 to 20 cm depth after six years (Tanner et al., 2016). The increase in soil C content with litter addition occurred despite an apparent priming effect of added litter on soil CO₂ fluxes (Sayer et al., 2011). Similar to our $\pm 30\%$ change in bulk soil C content with litter addition or removal, two years of litter manipulation in a Costa Rican forest on Ultisols also changed bulk soil C concentrations by approximately $\pm 30\%$ (Leff et al., 2012). In contrast, a review of litter

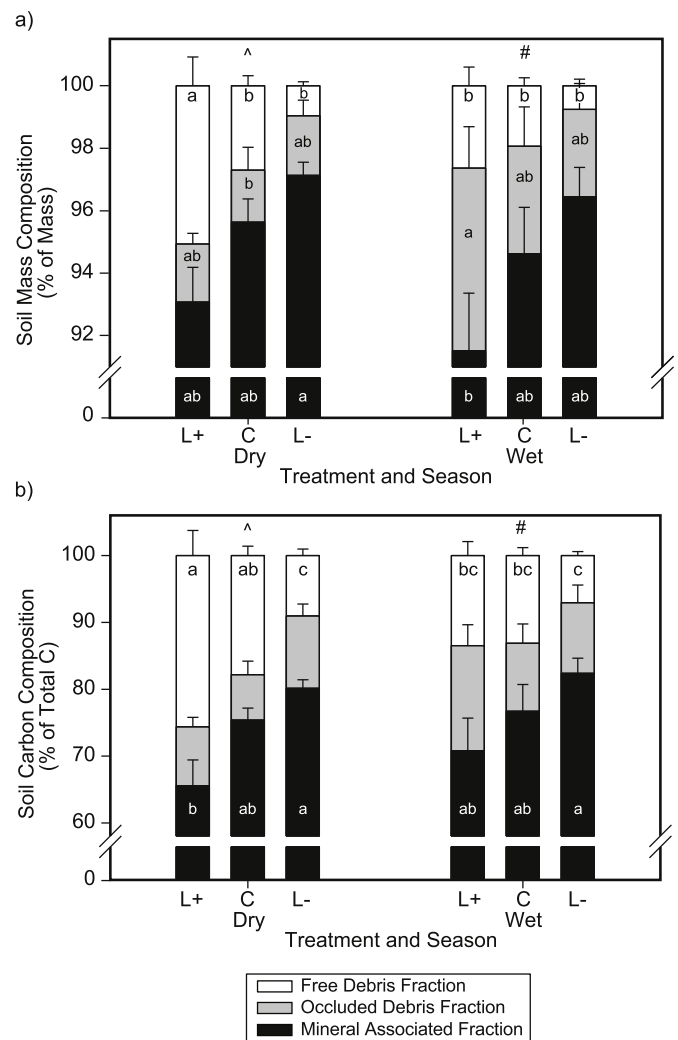


Fig. 5. The relative contributions of three density fractions to (a) total soil mass, and (b) total soil C are shown for litter addition (L+), control (C), and litter removal (L-) plots during a wet and dry season in Panama. Soil C fractions are presented as proportions of total (a) soil mass, and (b) soil C, adding up to 100%. Letters show significant differences among treatments and seasons for individual SOC fractions using Tukey HSD tests. ^ indicates that the overall contribution of the free debris fraction to total SOC and soil mass was greater during the dry versus wet season. # indicates that the total contribution of the occluded debris fraction to total SOC and soil mass was greater during the wet versus dry season, showing a seasonal contrast in the relative importance of these two soil fractions, both in terms of C content and total mass.

manipulations in temperate forests showed generally non-significant or small effects on bulk soil C content (Sayer, 2006). Our results, in conjunction with Leff et al. (2012), and the few tropical studies in the meta-analysis (Xu et al., 2013b), suggest that soil C stocks in strongly weathered tropical forest soils may be more sensitive to changes in NPP over shorter time periods than soils in temperate sites. Such a trend may reflect faster C cycling, greater overall C inputs to soils per year, and/or sorptive characteristics of the strongly weathered fine-textured soils common in humid tropical forests.

4.1. Elevated SOC across soil fractions with litter addition

The substantial accumulation of organic matter that we observed in the mineral-associated SOC fraction was somewhat surprising, since this soil fraction is commonly a relatively stable pool of long-term soil C storage (Schmidt et al., 2011). Greater mineral-associated SOC might be a response to the slightly higher soil pH in litter addition plots (Tanner

et al., 2016), since increased pH can mobilize DOC from organic matter (Whitehead et al., 1981), leaching C from litter into mineral soils where it can sorb to the relatively large number of binding sites common on clay surfaces in highly weathered tropical forest soils (Chorover and Sposito, 1995). Alternatively, DOC may have associated with other organic compounds already sorbed to mineral surfaces (Kleber et al., 2007), or to surfaces of very stable microaggregates ($< 1 \mu\text{m}$ diameter) (Torrent et al., 1990).

The low-density SOC fractions also accumulated in soils with litter addition, as expected, indicating that decomposition rates and other C losses did not accelerate to balance new litter inputs. The greater levels of occluded-debris SOC in litter addition plots could have resulted from organic matter incorporation into aggregate structures, particularly during formation and dissolution of aggregates over the course of rainfall seasonality (Dimoyiannis, 2009). In contrast to our results, a 50-year experiment in two temperate forests on silt-loams found that litter addition increased only the low-density SOC fractions, while litter removal reduced both low-density and mineral-associated SOC fractions (Lajtha et al., 2014). A better understanding of the ecosystem and soil characteristics that promote rapid accumulation of organic matter into stable mineral associations could help us better predict C cycle responses to global change across biomes.

4.2. Stability of C in SOC fractions: potential DOC leaching

As hypothesized, C incorporated into the mineral-associated soil fraction was subsequently relatively stable, as demonstrated by its low solubility and leaching potential. Similarly, added ^{15}N in a laboratory study was rapidly incorporated into the mineral-associated fraction of a range of soil orders from North and Central America sites, with subsequent low levels of solubility (Strickland et al., 1992). A desorption study using goethite and a C-poor mineral subsoil similarly showed $< 3\%$ desorption of organic matter when leaching solution was similar to sorption solution, and elevated desorption only under elevated ion concentrations or elevated pH (Kaiser and Zech, 1999). Thus, it appears that in one direction organo-mineral associations can be highly dynamic (e.g., rapid mineral sorption of organic compounds), but in the other direction, losses from this pool are more limited (i.e., not leachable as DOC), illustrating how this fraction can be a reservoir of long-term soil C storage. The longer-term stability of elevated mineral-associated SOC in litter addition plots merits further investigation.

Interestingly, the litter manipulation effect on DOC leaching as a proportion of extant SOC was different for the two low-density SOC fractions compared with the mineral-associated fraction. Litter removal versus addition plots had greater DOC leaching from low-density SOC fractions, but lesser DOC leaching from mineral-associated SOC fractions. This opposite treatment effect likely reflects the different physiochemical nature of organic matter in these different SOC fractions. Organic matter in the low-density fractions is comprised primarily plant or microbial macro tissues at some stage of decomposition, whereas the mineral-associated SOC fraction contains small organic compounds sorbed to mineral surfaces out of DOC. Thus, high levels of DOC leaching from low-density SOC fractions in litter removal plots likely resulted from the rapid dissolution of very decomposed macro tissues. In contrast, low levels of DOC leaching from the mineral-associated SOC fraction in litter removal plots likely resulted because the organo-mineral associations remaining after a decade of *in situ* leaching with rainwater were the most stably sorbed portion of mineral-associations.

4.3. Changes in the $\Delta^{14}\text{C}$ of mineral-associated SOC

Our radiocarbon data support growing evidence that only a portion of the mineral-associated SOC fraction is stable over the long term, whereas another portion is dynamic on decadal timescales. The relatively greater $\Delta^{14}\text{C}$ values of mineral-associated SOC in litter removal plots suggest decadal-scale retention of relatively older C, assuming

that the majority of C in surface soils originated from NPP during the past 60 years (Trumbore, 2000). In contrast, the lesser $\Delta^{14}\text{C}$ values in mineral-associated SOC in litter addition plots suggest an accumulation of relatively recent C inputs. Because $\Delta^{14}\text{C}$ is an integrated measure of all C included in the SOC fraction, the “modern” overall ages measured here do not preclude the presence of some much older C. For example, mineral-associated SOC fractions of surface soils in Puerto Rico had $\Delta^{14}\text{C}$ values of 74–81‰, similar to values here, and turnover times calculated using archived samples and a two-pool model showed that $> 30\%$ of the C had turnover times on the scale of millennia (Hall et al., 2015). The mineral-associated SOC fraction in general may be more accurately represented using a two-pool model (Torn et al., 2013). Thus, a portion of our mineral-associated SOC fraction is also likely much older than the overall radiocarbon values indicate, and the treatment effect we observed suggests that this older portion was the most stable component of mineral-associated SOC.

4.4. Changes in the organic chemistry of mineral-associated SOC

Mineral-associated SOC chemistry changed after a decade of litter removal, with increased dominance of alkyl, O-alkyl, and N-alkyl C, which is likely indicative of the relative stability of these C functional groups on mineral surfaces. The increased dominance of N-alkyl C groups in litter removal plot mineral associations points to the importance of proteins. Proteins have been put forth as a central component of stable mineral-associated SOC, because they sorb strongly to mineral surfaces and have hydrophobic outward-facing protons, leading to further associations with a wide range of organic compounds (Kleber et al., 2007). Several recent laboratory and field studies also provide support for the central role of microbial proteins in the formation of stable organo-mineral associations. One set of lab experiments showed rapid and preferential sorption of microbial proteins to mineral surfaces (Swenson et al., 2015), and another set showed that microbial biomass products dominated SOC accumulation in model soils (Kallenbach et al., 2016). In the field, a European forest litter decomposition experiment demonstrated that C from added litter became stably associated with mineral surfaces after incorporation into microbial biomass (Hatton et al., 2012), and an isotope labeling experiment in temperate grassland soils showed that soluble C compounds from litter were stored in SOC after microbial uptake (Cotrufo et al., 2015). In our study, the lower C:N ratios we observed in mineral-associated SOC with litter removal also suggest more advanced stage of microbial processing (Baisden et al., 2002), and/or greater protein content. Together, these results support growing evidence that microbial biomass products like proteins play a dominant role in the formation of stable, mineral-associated SOC.

Other C groups have also been posited as playing a role in long-term, stable C retention in soils. In particular, aromatic and phenolic compounds like lignin, as well as alkyl C, representative of waxes and other lipids, are preferentially preserved in soils (Baldock et al., 1992, 1997; De Marco et al., 2012). More generally, there is growing consensus that few, if any, organic compounds are retained in soils over the long-term based solely on their chemical structure (i.e. without some form of physical protection like sorption to minerals) (Schmidt et al., 2011) (Lehmann and Kleber, 2015). For aromatic-rich compounds, which can be lost relatively quickly from soils in field experiments (Gleixner and Poirier, 2001), desorption experiments suggest that the sometimes observed preferential retention of compounds like lignin may occur because aromatics can bind more strongly to mineral surfaces than other compounds in some cases (Kaiser and Zech, 1999; Kaiser, 2003). In our data, we saw no evidence for long-term retention of aromatic C in the mineral-associated SOC fraction. Also, the negative relationship between $\Delta^{14}\text{C}$ and phenolic C in mineral-associated SOC indicated that phenolics contributed a relatively younger, more dynamic portion of this SOC fraction. We did see increased dominance of alkyl C with litter removal, as expected. Litter chemistry can have a

strong influence on SOC chemistry (Kögel-Knabner, 2002), so the observed pattern may reflect the waxy, tough character of canopy foliage in these Panamanian forests (Coley, 1983). Alternately, alkyl C in soils can be strongly related to soil microbial biomass, as observed in subtropical plantations in China (Wang et al., 2015). Here, the increased dominance of alkyl C in litter removal plots was in mineral-associated SOC, suggesting that even this relatively recalcitrant C group depended on mineral associations for protection from decomposition.

In contrast to alkyl C, O-alkyl C, representative of carbohydrates like cellulose, is generally lost from litter quickly during microbial decomposition (Preston et al., 2009). Thus, the ratio of alkyl:O-alkyl C has been used to measure the extent of microbial processing of organic matter in litter and soil (Baldock et al., 1997; LaRowe et al., 2012; Keiluweit et al., 2016). It was therefore surprising that our litter removal plots contained greater proportions of both alkyl and O-alkyl C in mineral-associated SOC, and significantly lower soil alkyl:O-alkyl ratios relative to control plots. Also, the positive relationship between $\Delta^{14}\text{C}$ and O-alkyl C indicated that O-alkyls contributed a relatively older, more stable portion of mineral-associated SOC. Although unusual, preferential retention of O-alkyl C has been observed in some other forests, particularly for plant litters with high alkyl:O-alkyl starting ratios (e.g. waxy leaves), and resistant O-alkyl C compounds (e.g. from tannins) (Almendros et al., 2000). Also, cell wall polymers in fungal and bacterial biomass (e.g., chitin and peptidoglycan) are comprised largely of O-alkyl C plus proteins. Thus, the preferential retention of O-alkyl C that we observed with litter removal may indicate a particularly resistant carbohydrate group from plant litter. Together with the N-alkyl data (above), however, it seems likely that the increased O-alkyl C following litter removal indicates the predominance of microbial biomass products in mineral-associated SOC.

Overall, our data indicate that most plant-derived compounds, of which phenolic and aromatic C are indicative, are not retained in mineral associations over the long-term in humid tropical forest soils. Rather, microbial biomass compounds like proteins and cell wall polymers (containing O-alkyl and N-alkyl C groups), as well as lipids (alkyl C), appear to be preferentially retained on mineral surfaces in these soils on a decadal timescale. Our ^{13}C NMR data also suggest that a revised ratio of (alkyl C + O-alkyl C + N-alkyl):(aromatic + phenolic), may be a useful indicator of the biochemical stability of SOC in strongly weathered tropical forest soils.

4.5. Different seasonal effects across SOC fractions

Our data indicate that dry-season declines in mineral-associated and occluded-debris SOC explain an observed overall decrease in bulk soil C stocks during the dry season at these sites (Turner et al., 2015). The wet season increase in mineral-associated SOC, particularly with litter addition, may indicate the importance of rainfall leaching DOC from litter downward and subsequent DOC sorption to mineral surfaces (Qualls et al., 2002). The wet-season increase in occluded-debris SOC could be related to elevated formation and dispersal of macro aggregates (2–4.75 mm diameter), which is common during wet periods in seasonally dry sites (Bach and Hofmockel, 2016). In contrast, the wet-season decline in free-debris SOC likely reflects a two-fold increase in forest floor decomposition rates during the wet season in these Panamanian forests relative to the dry season (Wieder and Wright, 1995). Overall, our data suggest a seasonal transfer of SOC between fractions, with added litter incorporated into free-debris space during the dry season, and then possibly transferred to occluded-debris and/or mineral associated fractions during the subsequent wet season, along with C from new litter.

5. Conclusion

This study demonstrates that the physical and biochemical nature of soil C stocks are sensitive to changes in tropical forest NPP with global

change. Most notably, the relatively stable mineral-associated SOC fraction changed markedly following a decade of litter manipulation. Litter addition promoted the accumulation of C into relatively stable organo-mineral associations (i.e. not leachable as DOC), suggesting that strongly weathered tropical soils have the capacity to store more C if tropical forest NPP increases. The most stable portion of mineral-associated SOC included lipids like waxes (alkyl C), and microbial products like proteins and cell walls (N-alkyl and O-alkyl C). In contrast, plant-derived compounds, characterized by aromatic and phenolic C, formed a more dynamic portion of mineral-associated SOC, demonstrating that these compounds are less important than N-containing compounds for long-term soil C storage in strongly weathered tropical soils. Free-debris SOC accumulated during the dry season, whereas occluded-debris and mineral-associated SOC increased during the wet season, promoting greater bulk soil C content during the wet season. Thus, a change in the duration or severity of the dry season may interact with changes in tropical forest NPP to alter soil C storage in tropical forests. Overall, our findings show that changes in tropical forest NPP will alter the quantity, stability, and biochemical character of soil C stocks.

Acknowledgements

Funding was provided by DOE Office of Science TER-BER Early Career Research Program grant #DE-SC0015898, National Science Foundation GSS grant #BCS-1437591, and a Smithsonian Tropical Research Institute Short-Term Fellowship to D.F. Cusack. W.C. Hockaday acknowledges instrumentation and facilities support from NSF EAR-IF grant #1132124. We thank J. Rodriguez, W. Perez, D. Agudo, D. Ashdown, T. Hrast, T. Johnson, and T. McCleery for assistance. Helpful comments on the manuscript were provided by Dr. P. Sollins.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.soilbio.2018.06.005>.

References

- Almendros, G., Dorado, J., Gonzalez-Vila, F.J., Blanco, M.J., Lankes, U., 2000. C-13 NMR assessment of decomposition patterns during composting of forest and shrub biomass. *Soil Biology and Biochemistry* 32, 793–804.
- Bach, E.M., Hofmockel, K.S., 2016. A time for every season: soil aggregate turnover stimulates decomposition and reduces carbon loss in grasslands managed for bioenergy. *Global Change Biol. Bioenergy* 8, 588–599.
- Baisden, W.T., Amundson, R., Cook, A.C., Brenner, D.L., 2002. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Global Biogeochemical Cycles* 16.
- Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997. Assessing the extent of decomposition of natural organic materials using solid-state C-13 NMR spectroscopy. *Australian Journal of Soil Research* 35, 1061–1083.
- Baldock, J.A., Oades, J.M., Waters, A.G., Peng, X., Vassallo, A.M., Wilson, M.A., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR Spectroscopy. *Biogeochemistry* 16, 1–42.
- Boisier, J.P., Ciais, P., Ducharme, A., Guimberteau, M., 2015. Projected strengthening of Amazonian dry season by constrained climate model simulations. *Nature Climate Change* 5 656–+.
- Chorover, J., Spósito, G., 1995. Surface-charge characteristics of kaolinitic tropical soils. *Geochimica et Cosmochimica Acta* 59, 875–884.
- Clark, D.A., Clark, D.B., Oberbauer, S.F., 2013. Field-quantified responses of tropical rainforest aboveground productivity to increasing CO₂ and climatic stress, 1997–2009. *J. Geophys. Res.-Biogeosci.* 118, 783–794.
- Coley, P.D., 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* 53, 209–233.
- Cotrufu, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, A.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience* 8 776–+.
- Cusack, D., Markesteijn, L., Condit, R., Lewis, O., Turner, B., 2018. Soil carbon stocks in tropical forests of Panama regulated by base cation effects on fine roots. *Biogeochemistry* 137, 253–266.
- Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011a. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92, 621–632.
- Cusack, D.F., Silver, W.L., Torn, M.S., McDowell, W.H., 2011b. Effects of nitrogen

- additions on above- and belowground carbon dynamics in two tropical forests. *Biogeochemistry* 104, 203–225.
- De Marco, A., Spaccini, R., Vittozzi, P., Esposito, F., Berg, B., De Santo, A.V., 2012. Decomposition of black locust and black pine leaf litter in two coeval forest stands on Mount Vesuvius and dynamics of organic components assessed through proximate analysis and NMR spectroscopy. *Soil Biology and Biochemistry* 51, 1–15.
- Dimoyiannis, D., 2009. Seasonal soil aggregate stability variation in relation to rainfall and temperature under Mediterranean conditions. *Earth Surface Processes and Landforms* 34, 860–866.
- Feng, X., Porporato, A., Rodriguez-Iturbe, I., 2013. Changes in rainfall seasonality in the tropics. *Nature Climate Change* 3, 811–815.
- Gelinas, Y., Baldock, J.A., Hedges, J.I., 2001. Demineralization of marine and freshwater sediments for CP/MAS ^{13}C NMR analysis. *Organic Geochemistry* 32, 677–693.
- Glaser, B., Balashov, E., Haumaier, L., Guggenberger, G., Zech, W., 2000. Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic Geochemistry* 31, 669–678.
- Gleixner, G., Poirier, A., 2001. Molecular turnover rates of soil organic matter. Abstracts of Papers American Chemical Society 221 U519–U519.
- Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. *Australian Journal of Soil Research* 32, 285–309.
- Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1995. Structural and dynamic properties of soil organic matter as reflected by ^{13}C natural abundance, pyrolysis mass spectrometry and solid state ^{13}C NMR spectroscopy in density fractions of an oxisol under forest and pasture. *Australian Journal of Soil Research* 33, 59–76.
- Hall, S.J., McNicol, G., Natak, T., Silver, W.L., 2015. Large fluxes and rapid turnover of mineral-associated carbon across topographic gradients in a humid tropical forest: insights from paired ^{14}C analysis. *Biogeochemistry* 12, 2471–2487.
- Hatton, P.J., Kleber, M., Zeller, B., Moni, C., Plante, A.F., Townsend, K., Gelhaye, L., Lajtha, K., Derrien, D., 2012. Transfer of litter-derived N to soil mineral-organic associations: evidence from decadal N-15 tracer experiments. *Organic Geochemistry* 42, 1489–1501.
- Hockaday, W.C., Masiello, C.A., Randerson, J.T., Smernik, R.J., Baldock, J.A., Chadwick, O.A., Harden, J.W., 2009. Measurement of soil carbon oxidation state and oxidative ratio by ^{13}C nuclear magnetic resonance. *J. Geophys. Res.-Biogeosci.* 114.
- Holdridge, L., Grenke, W., Hatheway, W., Liang, T., Tosi, J., 1971. *Forest environments in Tropical Life Zones*. Pergamon Press, New York.
- Jobbagy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10, 423–436.
- Kaiser, K., 2003. Sorption of natural organic matter fractions to goethite ($\alpha\text{-FeOOH}$): effect of chemical composition as revealed by liquid-state C-13 NMR and wet-chemical analysis. *Organic Geochemistry* 34, 1569–1579.
- Kaiser, K., Zech, W., 1999. Release of natural organic matter sorbed to oxides and a subsoil. *Soil Science Society of America Journal* 63, 1157–1166.
- Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications* 7, 10.
- Keiluweit, M., Nico, P.S., Kleber, M., Fendorf, S., 2016. Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? *Biogeochemistry* 127, 157–171.
- Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Mineral-organic associations: formation, properties, and relevance in soil environments. In: Sparks, D.L. (Ed.), *Advances in Agronomy*. 130. Elsevier Academic Press Inc, San Diego, pp. 1–140.
- Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85, 9–24.
- Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* 34, 139–162.
- Lajtha, K., Townsend, K.L., Kramer, M.G., Swanston, C.W., Bowen, R.D., Nadelhoffer, K., 2014. Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems. *Biogeochemistry* 119, 341–360.
- LaRowe, D.E., Dale, A.W., Amend, J.P., Van Cappellen, P., 2012. Thermodynamic limitations on microbially catalyzed reaction rates. *Geochimica et Cosmochimica Acta* 90, 96–109.
- Leff, J.W., Wieder, W.R., Taylor, P.G., Townsend, A.R., Nemergut, D.R., Grandy, A.S., Cleveland, C.C., 2012. Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest. *Global Change Biology* 18, 2969–2979.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528, 60–68.
- Leigh, E.G., 1999. *Tropical Forest Ecology*. Oxford University Press, Oxford.
- Leigh Jr., E.G., Rand, A.S., Windsor, D.W., 1996. *The Ecology of a Tropical Forest*, second ed. Smithsonian Press, Washington DC.
- Lewis, S.L., Lloyd, J., Sitch, S., Mithard, E.T.A., Laurance, W.F., 2009. Changing ecology of tropical forests: evidence and drivers. *Annual Review of Ecology Evolution and Systematics* 40, 529–549.
- Li, Y.F., Chen, N., Harmon, M.E., Li, Y., Cao, X.Y., Chappell, M.A., Mao, J.D., 2015. Plant species rather than climate greatly alters the temporal pattern of litter chemical composition during long-term decomposition. *Scientific Reports* 5, 13.
- Marin-Spiotta, E., Silver, W., Swanston, C., Ostertag, R., 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. *Global Change Biology* 15, 1584–1597.
- Marin-Spiotta, E., Swanston, C.W., Torn, M.S., Silver, W.L., Burton, S.D., 2008. Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. *Geoderma* 143, 49–62.
- Min, K., Freeman, C., Kang, H., Choi, S.U., 2015. The regulation by phenolic compounds of soil organic matter dynamics under a changing environment. *BioMed Res. Int.* 11.
- Moni, C., Derrien, D., Hatton, P.J., Zeller, B., Kleber, M., 2012. Density fractions versus size separates: does physical fractionation isolate functional soil compartments? *Biogeochemistry* 9, 5181–5197.
- Nemani, R.R., Keeling, C.D., Hashimoto, H., Jolly, W.M., Piper, S.C., Tucker, C.J., Myrneni, R.B., Running, S.W., 2003. Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *Science* 300, 1560–1563.
- Preston, C.M., Nault, J.R., Trofymow, J.A., 2009. Chemical changes during 6 Years of decomposition of 11 litters in some canadian forest sites. Part 2. C-13 abundance, solid-state C-13 NMR spectroscopy and the meaning of "lignin". *Ecosystems* 12, 1078–1102.
- Qualls, R.G., Haines, B.L., Swank, W.T., Tyler, S.W., 2002. Retention of soluble organic nutrients by a forested ecosystem. *Biogeochemistry* 61, 135–171.
- Sayer, E.J., 2006. Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biological Reviews* 81, 1–31.
- Sayer, E.J., Heard, M.S., Grant, H.K., Marthews, T.R., Tanner, E.V.J., 2011. Soil carbon release enhanced by increased tropical forest litterfall. *Nature Climate Change* 1, 304–307.
- Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1997. Improvement of ^{13}C and ^{15}N CP/MAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. *European Journal of Soil Science* 48, 319–328.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56.
- SoilSurveyStaff, 1999. *Soil Taxonomy: a Basic System of Soil Classification for Making and Interpreting Soil Surveys*, second ed. (Lincoln, NE).
- Sollins, P., Glassman, C.A., Paul, E.A., Swanston, C., Lajtha, K., Heil, J.W., Elliott, E.T., 1999. Soil carbon and nitrogen: pools and fractions. In: Sollins, P. (Ed.), *Standard Soil Methods for Long-term Ecological Research*, first ed. Oxford University Press, New York, pp. 89–105.
- Sollins, P., Gregg, J.W., 2017. Soil organic matter accumulation in relation to changing soil volume, mass, and structure: concepts and calculations. *Geoderma* 301, 60–71.
- Sollins, P., Swanston, C., Kleber, M., Filley, T., Kramer, M., Crow, S., Caldwell, B.A., Lajtha, K., Bowden, R., 2006. Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. *Soil Biology and Biochemistry* 38, 3313–3324.
- Strickland, T.C., Sollins, P., Rudd, N., Schimel, D.S., 1992. Rapid stabilization and mobilization of ^{15}N in forest and range soils. *Soil Biology and Biochemistry* 24, 849–855.
- Swanston, C.W., Torn, M.S., Hanson, P.J., Southon, J.R., Garten, C.T., Hanlon, E.M., Gano, L., 2005. Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. *Geoderma* 128, 52–62.
- Swenson, T.L., Bowen, B.P., Nico, P.S., Northen, T.R., 2015. Competitive sorption of microbial metabolites on an iron oxide mineral. *Soil Biology and Biochemistry* 90, 34–41.
- Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis: Part 3: Chemical Methods*. Soil Science Society of America, Madison, WI, pp. 1011–1069.
- Tan, Z.H., Cao, M., Yu, G.R., Tang, J.W., Deng, X.B., Song, Q.H., Tang, Y., Zheng, Z., Liu, W.J., Feng, Z.L., Deng, Y., Zhang, J.L., Liang, N.S., Zhang, Y.P., 2013. High sensitivity of a tropical rainforest to water variability: evidence from 10 years of inventory and eddy flux data. *J. Geophys. Res. Atmos.* 118, 9393–9400.
- Tanner, E.V.J., Sheldrake, M., Turner, B.L., 2016. Changes in soil carbon and nutrients following six years of litter removal and addition in a tropical semi-evergreen rain forest. *Biogeochemistry* 13, 6183–6190.
- Torn, M.S., Kleber, M., Zavaleta, E.S., Zhu, B., Field, C.B., Trumbore, S.E., 2013. A dual isotope approach to isolate soil carbon pools of different turnover times. *Biogeochemistry* 10, 8067–8081.
- Torn, M.S., Trumbore, S.E., Chadwick, O.A., Vitousek, P.M., Hendricks, D.M., 1997. Mineral control of soil organic carbon storage and turnover. *Nature* 389, 170–173.
- Torrey, J., Barron, V., Schwertmann, U., 1990. Phosphate adsorption and desorption by goethites differing in crystal morphology. *Soil Science Society of America Journal* 54, 1007–1012.
- Trumbore, S., 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications* 10, 399–411.
- Trumbore, S.E., 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. *Global Biogeochemical Cycles* 7, 275–290.
- Trumbore, S.E., Zheng, S.H., 1996. Comparison of fractionation methods for soil organic matter ^{14}C analysis. *Radiocarbon* 38, 219–229.
- Turner, B.L., Yavitt, J.B., Harms, K.E., Milton, G.N., Wright, S.J., 2015. Seasonal changes in soil organic matter after a decade of nutrient addition in a lowland tropical forest. *Biogeochemistry* 123, 221–235.
- Vincent, A.G., Turner, B.L., Tanner, E.V.J., 2010. Soil organic phosphorus dynamics following perturbation of litter cycling in a tropical moist forest. *European Journal of Soil Science* 61, 48–57.
- Vogel, J.S., Southon, J.R., Nelson, D.E., Brown, T.A., 1984. Performance of catalytically condensed carbon for use in accelerator mass-spectrometry. *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms* 233, 289–293.
- von Lutzow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European Journal of Soil Science* 57, 426–445.
- Wagai, R., Mayer, L.M., Kitayama, K., 2009. Nature of the "occluded" low-density fraction in soil organic matter studies: a critical review. *Soil Science & Plant Nutrition* 55,

- 13–25.
- Wang, H., Liu, S.R., Chang, S.X., Wang, J.X., Shi, Z.M., Huang, X.M., Wen, Y., Lu, L.H., Cai, D.X., 2015. Soil microbial community composition rather than litter quality is linked with soil organic carbon chemical composition in plantations in subtropical China. *Journal of Soils and Sediments* 15, 1094–1103.
- Whitehead, D.C., Dibb, H., Hartley, R.D., 1981. Extractant pH and the release of phenolic compounds from soils, plant roots and leaf litter. *Soil Biology and Biochemistry* 13, 343–348.
- Wieder, R.K., Wright, S.J., 1995. Tropical forest litter dynamics and dry season irrigation on Barro Colorado Island. *Panama Ecol.* 76, 1971–1979.
- Windsor, D.M., 1990. Climate and moisture variability in a tropical forest: long-term records from Barro Colorado island, Panama. *Smithsonian Contributions to the Earth Sciences* 29, 1–145.
- Xu, S., Liu, L.L., Sayer, E.J., 2013a. Variability of above-ground litter inputs alters soil physicochemical and biological processes: a meta-analysis of litterfall-manipulation experiments. *Biogeosciences* 10, 7423–7433.
- Xu, S., Lui, L., Sayer, E.J., 2013b. Variability of above-ground litter inputs alters soil physicochemical and biological processes: a meta-analysis of litterfall-manipulation experiments. *Biogeosciences* 10, 7423–7433.
- Yavitt, J.B., Harms, K.E., Garcia, M.N., Wright, S.J., He, F., Mirabello, M.J., 2009. Spatial heterogeneity of soil chemical properties in a lowland tropical moist forest, Panama. *Australian Journal of Soil Research* 47, 674–687.
- Yavitt, J.B., Wright, S.J., 2001. Drought and irrigation effects on fine root dynamics in a tropical moist forest, Panama. *Biotropica* 33, 421–434.